ONE DAY WORKSHOP

ON

Instrumental Techniques in Chemical Analysis

04 th Feb 2020

Organized By

DEPARTMENT OF CHEMISTRY

GOVERNMENT DEGREE COLLEGE, BELLAMPALLY

DIST. MANCHE

FORE WORD

This paper describes the design and implementation of Instrumental Technique in Chemical Analysis, to enhance B.Sc.students appreciation of practical knowledge of chemistry. The workshop is designed to gain the practical skills in chemistry. The students learnt the practical skills of instrumentation through hands on experience with facilitation of the resource person.

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Detection of purity of an organic compound by Thin Layer Chromatography (TLC)

Thin Layer chromatography (TLC) is an extremely valuable analytical technique in the organic lab. It provides a raid separation of compounds, and thereby gives an indication of the number and nature of the components of a mixture. TLC can also be used to identify compounds by comparison with known samples, to check the purity of a compound, or to monitor the progress of a reaction, an extraction, or a purification procedure.

This experiment will introduce you to the mechanics of TLC, and the chemical principles behind it. In the first part, you will separate the soluble components of spinach extract; in the second, you will analyze the compounds you separated by extraction in the first lab.

Principles of TLC. TLC is normally done on a small glass or plastic plate coated with a thin layer of a solid – the most common are silicon (SiO_2) or alumina (Al_2O_3). This is the stationary phase. The mobile phase is an organic solvent or solvent mixture. The sample mixture is applied near the bottom of the plate as a small spot, then placed in a jar containing a few ml of solvent. The solvent climbs up the plate by capillary action, carrying the sample mixture along with it. Each solubility in the mobile phase and the strength of its absorption to the stationary phase. When the solvent gets near the top of the plate, it is allowed to evaporate, leaving behind the components of the mixture at various distances from the point of origin. The ratio of the distance a compound moves to the distance the solvent moves is the R_f value (retention factor). This value is characteristic of the compound, the solvent, and the stationary phase.

(I). Selection of adsorbent:

Generally for TLC, the absorbent used is a thin layer of alumina or silica gel with a small amount of calcium sulphate to increase the strength of the layer. The adsorbent should satisfy the following conditions:

(a) It Should be insoluble in the solvent to be used for the separation.

(b) It should not react with the substances to be separated it should not act as a catalyst for their decomposition, rearrangement or isomerisation.

(c) It should be colorless.

(d) It should have uniform composition

The active solid adsorbent has a wide surface area having many polar sites that can reversibly adsorb small concentrations of substances by electrostatic forces of attraction. These are generally Vander Waal's forces, inductive forces, hydrogen bonding or the charge transfer depending upon the relative binding power of the components of the mixture.

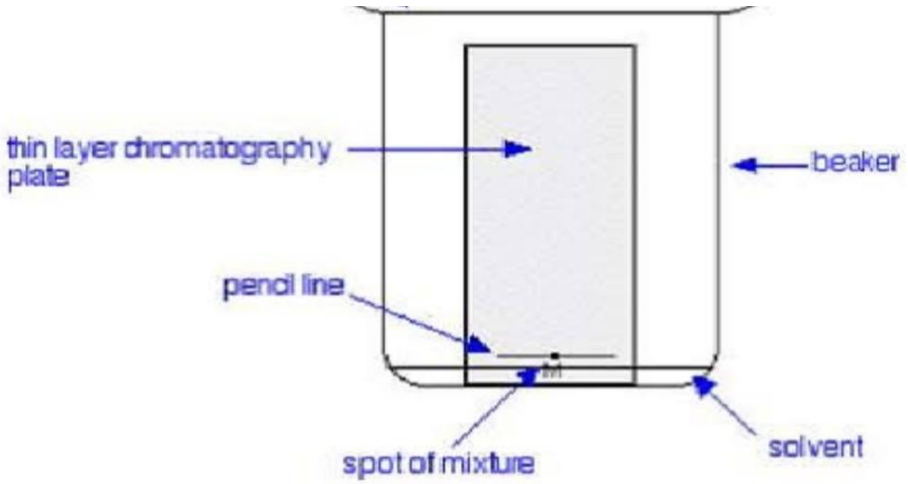
Separation of Binary Mixtures:

The strength of adsorption depends upon the strength of weak attractive forces between the components and the polar surface of the adsorbent. It depends upon the nature and the number of polar groups in the molecules of the substance. The decreasing order of polarity of different organic substances is given as:

Carboxylic acids> alcohols, phenols, amines, thiols, aldehydes, kentones, esters> organic halides> unsaturated hydrocarbons> saturated hydrocarbon…….

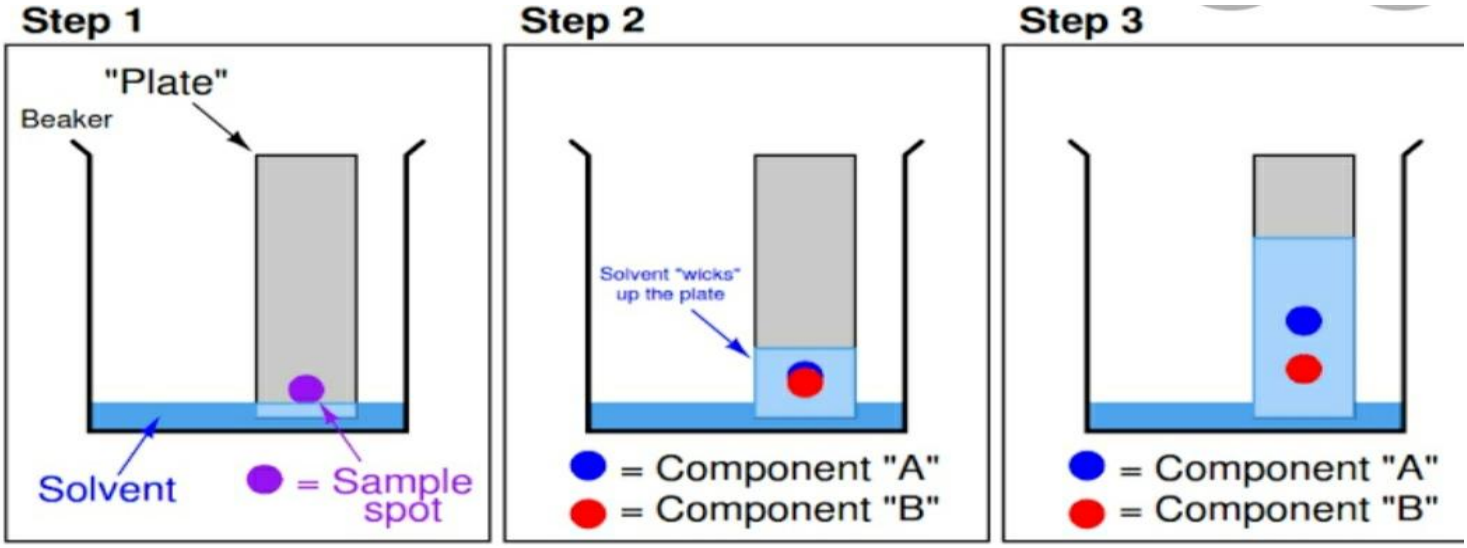
Preparation of the TLC plate

Handle TLC plates by the edges only, taking care not to touch the white surface with your fingers; Collect two TLC plates of dimensions 10 cm x 5 cm. Place the plates on a clean dry surface and using a pencil and a ruler draw a line 2.0 cm from the short edge. Press lightly with the pencil so as not to damage the silica layer. On this line, mark points starting 0.9 cm in from one side and then at 0.8 cm intervals to give 5 points. One point is for the unknown and the others are for the standards. Label the points lightly with pencil and include your initials at the top of the plates. Label one plate A and the other plate B.

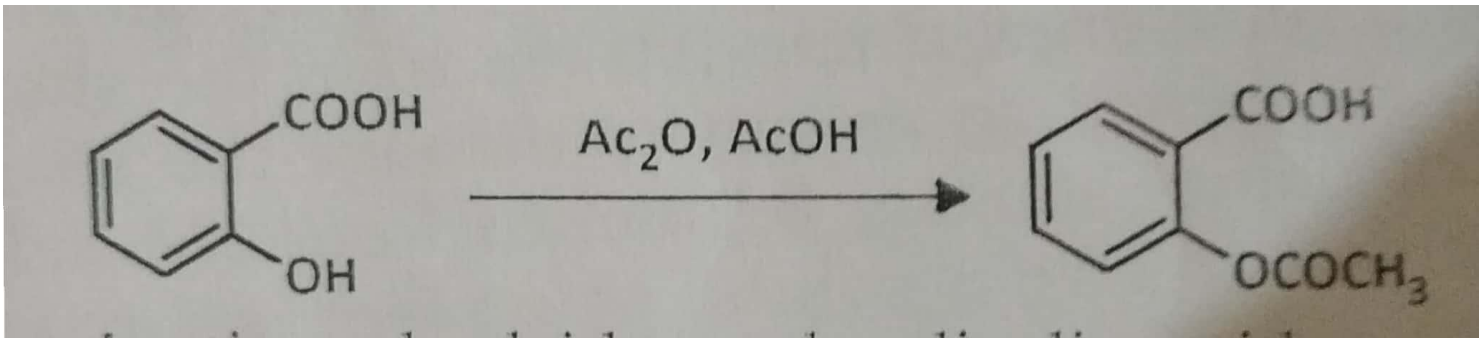


Selection of solvent:

Based on the polarity order, we have to choose the the solvent.

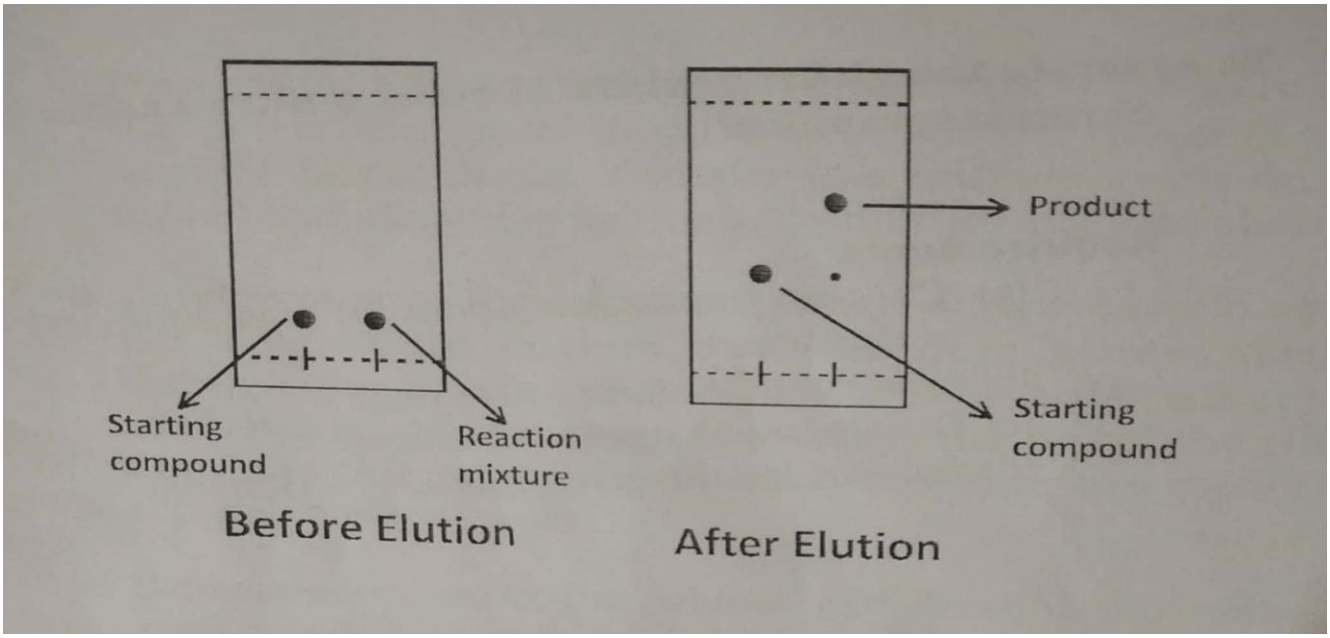


Checking the purity of the compound Acylation of Salicylic and



Acetic anhydride and salicylic acid react to produce acetylsalicylic acid and acetic acid; Sulfuric acid is used as a catalyst. The excess acetic anhydride is then decomposed with water to form acetic acid. Acetylsalicyclic acid is not very soluble in cold water (- 0.25 g per 100 ml) and consequently it can be isolated by diluting the reaction mixture with water and filtering off the solid product.

Working in a fume hood, take acetic anhydride (5.0 mL) in a clean, dry, 10 mL measuring cylinder and pour it into the flask containing the salicylic acid in such a way as to wash down any crystals that may have adhered to the walls of the flask. While swirling the flask, carefully add 3 drops of concentrated sulfuric acid. (Caution! Corrosive – avoid contact with skin and clothing.) Cover the flask with a small watch glass to prevent condensation of water inside the flask during heating on the steam bath. Heat the flask on a steam bath for 15 minutes.



Take a sample of the reaction mixture with a capillary and make a spot on the TLC plate. And make another spot of the starting compound in the same TLC plate. And make another spot of the starting compound in the same TLC plate. Now elute the reaction mixture in appropriate solvent. We can observe the two spots in the reaction mixture spot. Stop the experiment when the product spot is predominantly appeared in the TLC plate. In this way we can check the purity of the compound by using TLC.

Separation of 2,4 – Dinitrophenylhydrazones

The 2,4 – Dinitrophenylhydrazones derivatives of Acetoney 2 – buta none colored and can be separated and easily visualized on TLC plate.

Procedure:

Coat a 10 cm plate using silica gel. Prepare a solution in chloroform or dioxane by mixing 10 mg of each hydra zone. Spot the TLC plate with this mixture solution as well as apply one spot each of the individual pure hydro zone for comparison. Allow the spots to dry and then place the plate in a developing chamber containing Toluene petroleum ether (3:1) solvent. Develop the chromatogram. Remove the plate from the chamber, mark the position of the solvent front and the colored spots. Allow the plate to dry. Estimate the R_f values and identify the separation of the mixture with the help of the standard spots.

To separate the given mixture of o – and p – nitro aniline chromatographically.

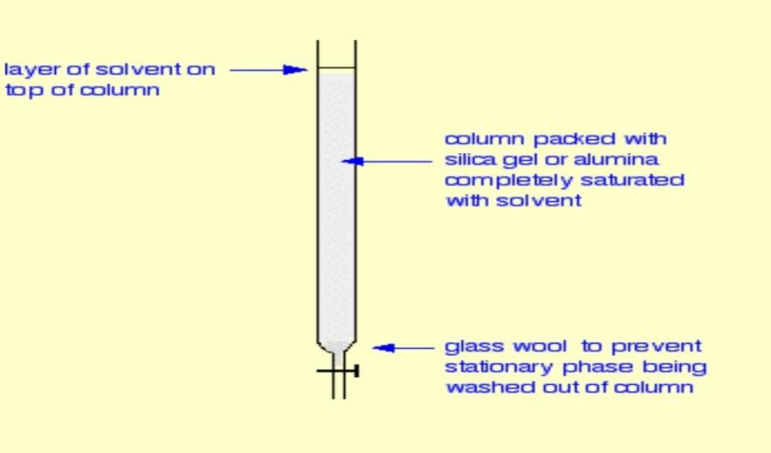
Requirements

(a) Column (preferable about 50 cms, long and about 3 cms in diameter)

(b) Adsorbent – Active alumina.

(c) Development agent – Benzene

(d) Solvent for the given mixture – Benzene



Procedure: Preparation of the column

(i) Mix 150 gms, of active alumina with sufficient quantity of dry benzene so as to form slurry.

(ii) Take a narrow edge glass tube approximately 50 cm. In length and 3 cm in diameter (fig:5.5) wash it carefully with chromic acid followed by distilled water and alcohol. Insert a wad of glass wool within the narrow edge of the tube to protect the absorbent to flow out. Pour the slurry of alumina into the tube carefully and allow it to settle. Clamp it and a dropping funnel just above the top of the column in a stand. Open the pinch cock of the column and allow benzene to run down slowly so that the column settles. The top of the column should be kept immersed in the solvent. Cover the top of the column with a filter paper if necessary.

(a) Preparation and application of the sample – Dissolve about 0.5 gm of the given mixture in sufficient quantity of dry benzene (about 35 ml) Transfer this solution to the dropping funnel and allow this solution to enter the column slowly.

(b) Development of the column – When all the sample solution has entered the column, run the pure solvent (benzene) through the column immediately. Collect the solvent from the bottom and run it again through the top of the column. Continue this process until there appears two distinct and separate yellow layers.

(c) Separation – wash the column with fresh dry benzene until each band is eluted separately, o-nitroaniline emerges prior top-isomer.

Concentrate each elute in a boiling water bath to about 20-25 ml and then pour them on watch glasses to evaporate the rest of the benzene.

Purify the separated products.

Result:

Separated products	:	MP (observed)
o-nitroaniline	:	72^0C
p-nitroaniline	:	148^0C

Precautions:

(i) The wad of glass wool should be inserted carefully. It should not adhere to the sides of the column.

(ii) Pour the slurry carefully so that it may not have air bubbles.

(iii) The top of the column should always remain immersed in the solvent.

DETERMINATION OF CONCENTRATION OF HC1

CONDUCTMETRICALLY USING STANDARD HCL SOLUTION

AIM: To determine the concentration of a given strong acid by conduct metric titration.

APPARATUS: conductivity bridge, conductivity cell, burette and pipettes

CHEMICALS REQUIRED: Hydrochloric acid (in the range of 0.01 M), sodium hydroxide (0.1 M) – both prepared in conductivity water.

PRINCIPLE: When a strong acid, say HCl, is titrated with a strong base, NaOH, the following reaction takes place.

$$(H^+ + Cl) + (Na^+ + OH^-) \rightarrow Na^+ + Cl^- + H_2O$$

Thus, the highly conducting H^+ ions initially present in the solution are replaced by Na^+ ions having lower conductivity, Consequently, during the course of the reaction the conductance will be progressively decreasing. However, when excess alkali is added, due to the presence of more labile OH^- ions, the conductance will start increasing. Thus, at the equivalence point the conductance will be minimum.

PROCEDURE: Pipette out 40 ml of the give HCl solution into a clean conductivity cell of known cell const, take the standardized solution of NaOH in clean burette (Micro burette is prefereable) should be at least five times higher than that of the acid solution, so that the volume change would be minimal. Add the NaOH solution from the burette carefully, say just 0.5 ml each time. After each addition note down the conductance value. Continue the titration, till the two limbs of the curve are clearly traced.

Treatment of Data:

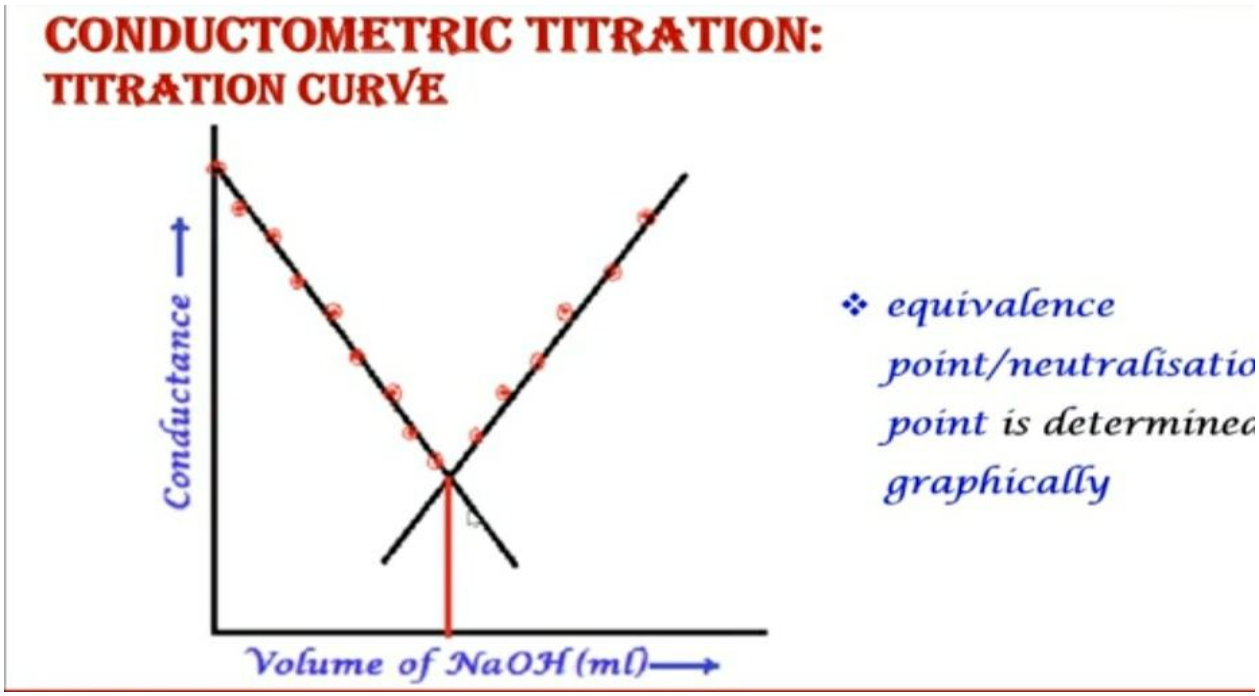
Cell constant = …… cm^{-1}

Volume of HCl = ……..ml

Strength of NaOH = …….. N (N NaOH)

Plot the conductance value against the corresponding volume of NaOH added. A curve with two limbs as shown in Fig 30 will be obtained. The point at which these two lines intersect corresponds to the volume of NaOH(V NaOH) required for exact neutralization of the acid.

Strength of the given HCl solution= $\frac{V\ NaOH x\ N\ NaOH}{20}$



Strong acid – strong base conduct metric titration graph.

Results:

(i) Volume of NaOH at equivalence point, V NaOH= ……. ml

(ii) Strength of the given strong acid = ……..N.

DETERMINATION OF CONCENTRATION OF ACETIC ACID CONDUCTMETRICALLY USING STANDARD NaOH SOLUTION

AIM: To determine the concentration of a given weak acid by conduct metric titration.

APPARATUS: Conductivity Bridge, conductivity cell, burette and pipettes.

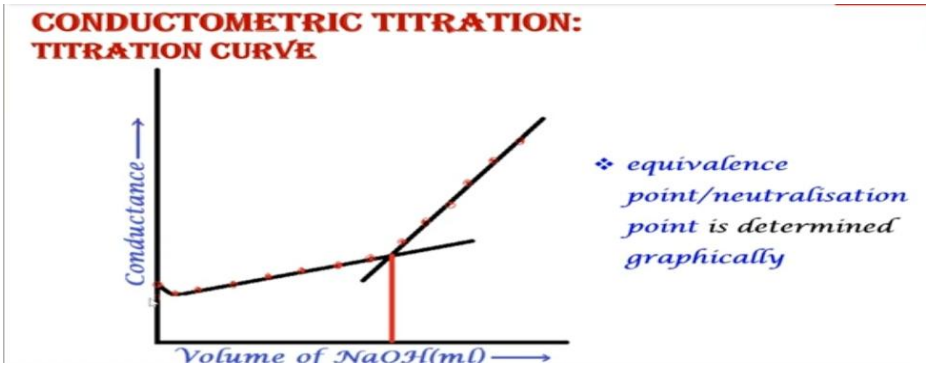
CHEMICALS REQUIRED: Acetic acid (in the range of 0.01 M), standard NaOH solution (In the range of 0.1 M) – both prepared in conductivity water.

PRINCIPLE: When a moderately weak acid, say Acetic acid, is titrated with a strong base like NaOH, the following reaction takes place.

$$CH_3COOH + NaOH \rightarrow CH_3COO^-Na^+ + H_2O$$

Acetic acid, being weak, ionizes only partially. So, the conductance for the solution initially will be low. On adding a very small amount of alkali, CH_3COONa will be formed. However, the common ion (acetate ion) will suppress the ionization of acetic acid: hence, there may be a further decrease in the conductance initially. However, this initial suppression is overcome at higher alkali concentrations and the conductance starts progressively increasing. When the acid is completely neutralized, further addition of alkali will result in the presence of highly conducting Na^+ and OH^- ions in the solution, associated with asteep increase in conductance. This break point will give the equivalence point.

PROCEDURE: Pipette out 20 ml of the give acetic acid solution into a clean conductivity cell of known cell const. Add the standardized NaOH solution taken in burette (preferably in a micro burette) in small aliquots, say 0.1 ml, to the acid. After each addition , stir the contents well note down the resistance or conductance value. Continue the titration, till the two limbs of the curve are clearly traced.



Weak acid – strong base conducto metric titration graph

Treatment of Data:

Cell constant = ……cm^{-1}

Volume of the weak acid = 20 ml

Strength of NaOH = ……N(N_{NaOH})

Plot the conductance value against the corresponding volume of NaOH added. A curve with two limbs as shown in the (Fig 31) will be obtained, The point of intersection of these two lines corresponds to the volume of NaOH (V_{NaOH}) required for exact neutralization of the acid.

.: **strength of the given weak acid =** $\frac{V\ NaOH X N\ NaOH}{20}$

= ……N

RESULT:

(i) Volume of NaOH at equivalence point, V_{NaoH} = …….. ml

(ii) Strength of the given strong acid = ……N

DETERMINATION OF DISSOCIATION CONSTANT (Ka) OF ACETIC ACID BY CONDUCTIVITY MEASUREMENTS

AIM: To verify the Ostwald's dilution law for a given weak electrolyte and determine its dissociation constant.

Apparatus required: Conductivity Bridge, conductivity cell, 100 ml volumetric flasks and pipettes.

Chemicals required: 0.1 N acetic acid (or any weak electrolyte), conductivity water.

Principle: Weak electrolytes are only partially dissociated in solution. Hence, for such electrolytes the dissociation constant (K) is given by the Ostwald's dilution law as $K=C\alpha^2/(1-\alpha)$ where C is its molar concentration, and α is the degree of dissociation. The value of α is given the ratio of the equivalent conductivity of the electrolyte at a particular concentration to that at infinite dilution i.e. $\alpha=n/n0$ A However, in such case n0 may be determined only by the application of Kohlrausch's law of independent migration of ions.

Procedure:

Prepare different concentrations, say 0.05 , 0.02, 0.01, 0.05, 0.002 N of the given weak electrolyte by accurately diluting the given stock solution, in conductivity water. Using conductivity cell of known cell constant, measure the resistance for each of the above solutions. Calculate the specific conductivity and using no value for the electrolyte compute the dissociation constant, at the experimental temperature.

S.No	Conc.of CH_3COOH	Conductance	Specific Conductance k	Equivalent conductance $\gamma=(1000\times k)/c$	Degree of Dissociation $\alpha=\gamma/\gamma_\infty$	Dissociation constant $Ka= C\alpha^2/(1-\alpha)$
1	0.1					
2	0.05					
3	0.02					
4	0.01					
5	0.005					
3	0.002					

Result: The dissociation constant of acetic acid is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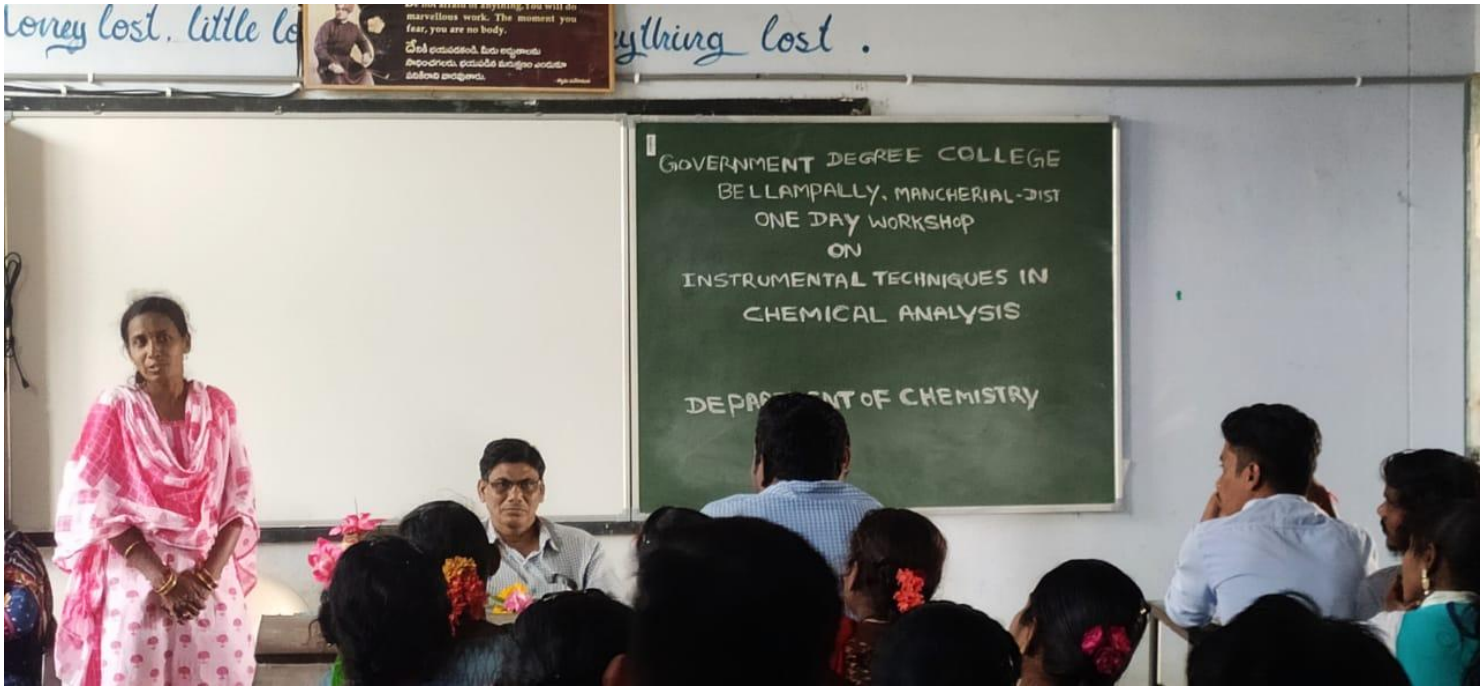
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Be not afraid of anything You will do
marvellous work. The moment you
fear, you are no body.
Experience is the best teacher.
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22	E. Manjula	BSC (BZC)	E. Manjula
23	A. Ramyakrishna	BZC.	A. Ramya.
24	P. Manasa	Bsc BZC	P. Manasa.
25	P. Jayanthi	B.S.C BZC	P. Jayanthi
26	S. Ramya sri	B.S.C (B.C.A)	S. Ramyasri
27	V. Ravi Teja	BSC (MPC)	V. Ravi Teja
28	P. Sai Teja	BSC (MPC)	P. Sai Teja.
29	B. Pinky	Bsc (MPC)	B. pinky
30	B. Sandeep.	BSC [MPC]	B. Sandeep.
31	D. pravalika	BSC [BZC]	D. pravalika
32	P. Anjamma	B.Sc [BZC]	P. Anjamma
33	E. Maheshwari	BSC (BZC]	E. Maheshwari
34	P. Manasa.	Bsc BZC	P. Manasa.
35	R. Laxmi	BSC. BZC	R. Laxmi
36	K. Nagamani	BSC (BZC)	K. Nagamani
37	D. Jyothi	Bsc (BZC)	D. Jyothi
38	T. Sravanthi	BSC M.P.C	T. Sravanthi
39	B. Rajitha	Bsc mpc	B. Rajitha
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